









# Evaluation of piglet birth weight on growth performance and qualitative and quantitative characteristics of carcasses of immunocastrated pigs

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**ABSTRACT** - The objective of this study was to determine the effect of birth weight on growth performance, carcass characteristics, and meat quality of immunocastrated male pigs. Seventy-two boars were assigned to three birth weight categories (BiW): light BiW pigs (LP: 0.98±0.083 kg BW; n = 24), normal BiW pigs (NP: 1.42±0.067 kg BW; n = 24), and heavy BiW pigs (HP: 1.85±0.096 kg BW; n = 24). For treatment design, we considered a weight range of two standard deviations (SD) in relation to the average population body weight (from 0.785 to 2.155 kg BW<sup>-1</sup>). The animals were housed in six pens with 12 animals per pen, with *ad libitum* access to water and feed throughout the study. In the growing and finishing phases, pigs were immunocastrated with two doses of vaccine at 112 and 161 days of age. The three BiW categories were different up to departure from the nursery (70 days). After this period, there was no difference between NP and HP in terms of BiW. Measurements showed that a low BiW reduced the loin eye area (9.4%), *longissimus thoracis* muscle depth (7.6%), and meat:fat ratio (21.6%). Backfat thickness (21.5%) and fat area (11.8%) were higher in LP as compared with the NP and HP categories. Light BiW pigs presented a lower weight steak (7.0%) and belly + rib (8.6%) compared with NP and HP, but did not differ within the LP group. Light BiW pigs required a greater lodging time (14 days), generating higher accumulated feed intake to reach the same slaughter BiW as the other categories. Growth performance and carcass characteristics are influenced by the birth weight (BiW) category. Light BiW pigs require more time and feed intake to reach the same slaughter weight. Birth weight has positive effects on meat quality and weight of commercial cuts.

**Keywords:** carcass quality, meat quality, pig production

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Received: March 18, 2020

Accepted: August 14, 2021

**How to cite:** Ceron, M. S.; Oliveira, V.; Moraes, P. O.; Muniz, H. C. M.; Brito, K. K.; Chimainski, M.; Krebs, G. and Kessler, A. M. 2021. Evaluation of piglet birth weight on growth performance and qualitative and quantitative characteristics of carcasses of immunocastrated pigs. Revista Brasileira de Zootecnia 50:e20200052. <https://doi.org/10.37496/rbz5020200052>

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## 1. Introduction

In pig production, success and survival capacity depends on the adoption of new technologies, such as the use of sows with high ovulation rates (Li et al., 2020). Hyper-prolific sows have a greater number of live piglets per year, thereby increasing productivity. However, farms with hyper-prolific

sows report an increase in the number of piglets with low birth weight (BiW) and nonuniform milk production rates (Rehfeldt et al., 2008; Paredes et al., 2014).

Low BiW pigs are predisposed to higher mortality rates in the early postnatal stages, as well as to changes in growth rate, feed efficiency, carcass characteristics, commercial cut yield, and muscle fiber morphometry (Rehfeldt et al., 2008; Alvarenga et al., 2013). Also, low BiW pigs are generally associated with a lower carcass quality and a greater unevenness of commercial cuts, resulting in economic losses for the producer and the slaughterhouse (Quiniou et al., 2002; Rehfeldt et al., 2008).

Due to the increasing use of immunocastration as an alternative to surgical castration, it is important to study influences of BiW on performance, carcass characteristics, and meat quality in this new sex category. In this context, the objective of this study was to determine the effect of BiW on growth performance, carcass characteristics, and meat quality of immunocastrated male pigs.

## 2. Material and Methods

### 2.1. Site, animals, and diets

The study was carried out in Santa Maria, Rio Grande do Sul, Brazil (29°41'29" S, 53°48'3" W, 139 m a.s.l.). This research on animals was conducted according to the Institutional Committee on Animal Use (CEUA) under case number 8688070716.

The study was divided into four phases: suckling, nursery, growing, and finishing. The suckling phase had a total of 195 male piglets (0.785 to 2.155 kg body weight (BW)) from birth-order sows with three or four farrowings (Agrocères × Danbred), of which 72 animals were selected and divided into three BiW categories (treatments): light BiW pigs (LP:  $0.98 \pm 0.083$  kg BW;  $n = 24$ ), normal BiW pigs (NP:  $1.42 \pm 0.067$  kg BW;  $n = 24$ ), and heavy BiW pigs (HP:  $1.85 \pm 0.096$  kg BW;  $n = 24$ ). For the group design, a weight range of two standard deviations in relation to the average BW of the population was considered. Piglets were not castrated and were kept with sows until weaning. From 10 days of age, creep feeding was provided.

In the nursery phase (days 29-69), piglets were housed in pens. Piglets from the same BiW category were housed together ( $n =$  two pigs per pen). Each pen measured  $1.2 \text{ m}^2$ , with slatted polyethylene floors, and were equipped with semiautomatic feeders and adjustable nipple-type drinkers. Animals were fed a standard three-phase corn-soybean meal-based nursery feeding program. Feeding phases comprised the following: pre-starter diet for pigs of 5-9 kg BW, starter 1 (9-15 kg BW), and starter 2 (15-30 kg BW). Diet was formulated according to the National Research Council (NRC, 2012) and provided *ad libitum*. Room temperature was maintained within the thermal comfort zone for each developmental phase while in nursery.

In the growing and finishing phases (from 70 days of age to slaughter), pigs were housed in six pens of  $15 \text{ m}^2$  each (12 pigs per pen), equipped with FIRE® and two nipple drinkers. Due to a communication failure between FIRE® and the computer (error 48 from Manual do Sistema e Software FIRE®; OSBORNE, 2014), data from one of the NP pens was lost. Pigs from the same BiW category were housed together in the pens and fed a standard four-phase corn-soybean meal-based growing-finishing feeding program with the following phases: grower 1 diet (24-50 kg BW), grower 2 (50-70 kg BW), finisher 1 (70-100 kg BW), and finisher 2 (100-140 kg BW). Diets were formulated to meet or exceed nutrient requirements (NRC, 2012). Grower diet 1 contained 1.09% digestible lysine (DL) and  $3,250 \text{ kcal kg}^{-1}$  metabolizable energy (ME); grower diet 2 had 1.01% DL and  $3,250 \text{ kcal kg}^{-1}$  ME; finisher diet 1 contained 0.81% DL and  $3,250 \text{ kcal kg}^{-1}$  ME; and finisher diet 2 had 0.75% DL and  $3,100 \text{ kcal kg}^{-1}$  ME. Room temperature was  $20 \text{ }^\circ\text{C}$  with relative humidity of 70%.

Pigs were immunocastrated according to the vaccination protocol suggested by the manufacturer. The first and second doses (Vivax®, Zoetis®) were applied (2 mL, subcutaneous) at week 16 (112 days) and 23 (161 days), respectively. The NP and HP were slaughtered at 25 weeks (174 days), while the LP were slaughtered at 27 weeks (188 days) of age. The rationale for different slaughtering ages is due the need to achieve the standard slaughter weight.

## 2.2. Performance measurements

Each animal was weighed individually on day 1 (birth), day 28 (weaning phase), day 70 (nursery phase), day 112 (growing phase), and day 174 (finishing phase). The following data were recorded for each phase: average daily gain (ADG), average daily feed intake (ADFI), and feed conversion rate (FCR).

## 2.3. Carcass characteristics and carcass and meat quality

Data on carcass characteristics and post-slaughter meat quality were estimated on the left half carcass: slaughter weight (SW), hot (HCW) and cold (CCW) carcass weight, carcass yield ( $CY = (CCW/SW) \times 100$ ), left half hot carcass weight (LHH), left half cold carcass weight (LHC), pH at 45 min (pH45) and 24 h (pH24) in the *longissimus thoracis* muscle, backfat thickness (BT) at the 10th rib, fat area (FA), loin eye area (LEA), *longissimus thoracis* muscle depth (LMD), carcass meat:fat ratio ( $M:F = LEA/FA$ ), yield of meat in the cooled carcass ( $YMCC = 65.92 - ((0.685 \times BT) + (0.094 \times LMD) - (0.026 \times CCW))$ ), amount of meat in the cooled carcass ( $QCC = 7.38 - (0.48 \times BT) + (0.059 \times LMD) + (0.525 \times HCW)$ ), water-holding capacity (WHC), cooking loss ( $CL = ((\text{defrosted sample weight} - \text{sample roasted weight}) \times 100)/\text{defrosted sample weight}$ ), shear force (SF), and firmness (FI).

To perform the histological analyses, approximately 5-mm portions of the *longissimus thoracis* muscle were removed from the muscle fiber in the transverse direction of slaughtered pigs (60 carcasses). The collected fragments were fixed in 10% buffered formalin, dehydrated with ethanol in increasing concentrations (30-100%), diaphanized, and embedded in paraffin.

From each muscle fragment, 3- $\mu\text{m}$  thick serial histological sections per sample were obtained with the aid of a Leica model RM 2125 RT microtome. The slides were examined using the Leica ICC50 HD optical microscope with a photographic camera attached to the 10X objective lens. From each cut, one image was scanned, resulting in a total of 300 analyzed images.

Morphometric analyses of total area and muscle fiber density were performed using Image Pro-Plus® software version 6.0, with an edge restriction and an area size of 3,000 to 20,000 pixels, after adjustment with Photoshop 13® software to remove coloration artifacts and improve visualization of muscle fibers (Dubowitz et al., 2013). For each sample, partial volume density of muscle fiber ( $V_v, \%$ ) was determined using stereological analysis via the M42 multifunctional test system (Wilson et al., 1990):

$$V_v = \Sigma P(\text{muscle fiber})/\Sigma P(\text{reference volume}) \times 100 \quad (1)$$

Subsequently, a 2.5-cm sample of the *longissimus thoracis* muscle (cross-section) was collected from the left half carcass of all pigs to perform a centesimal analysis. The amount of crude protein (CP), ether extract (EE), moisture (MO), and mineral matter (MM) were determined following methods recommended by the Association of Official Analytical Chemists (AOAC, 1999).

To determine weight and yield of primary cuts, the left half carcasses were sectioned into primary cuts: palette, shank, steak, and rib and belly. Each cut was individually weighed to determine cut yield ( $Y, \%$ ), using the following equation:

$$Y = (\text{cut weight} \times 100)/LCCW \quad (2)$$

## 2.4. Experimental design and statistical analysis

The experimental design was completely randomized. Three groups were analyzed: light, normal, and heavy BiW pigs. Each animal was considered an experimental unit. Variables were analyzed according to the following mathematical models:

$$Y_{ij} = \mu + \beta_i + \varepsilon_{ij} \quad (3)$$

in which  $Y_{ij}$  = dependent variable (performance and ultrasound),  $\mu$  = mean of the variable,  $\beta_i$  = fixed effect birth weight, and  $\varepsilon_{ij}$  = experimental error associated with the observation  $Y_{ij}$ .

$$Y_{ij} = \mu + T_i + W_j + \varepsilon_{ij}, \quad (4)$$

in which  $Y_{ij}$  = dependent variable (carcass characteristics, meat quality, and yield of primary cuts),  $\mu$  = mean of the variable,  $T_i$  = fixed effect BiW,  $W_j$  = slaughter weight effect, and  $\varepsilon_{ij}$  = residual effect.

Differences between the means in analyses of variance were compared by Tukey's test at a 5% significance level ( $P < 0.05$ ).

### 3. Results

At weaning, the differences ( $P < 0.05$ ) between the BW of LP vs NP, LP vs HP, and NP vs HP were 24, 36, and 16%, respectively (Table 1). At the end of the nursery phase (70 days), these differences reduced ( $P < 0.05$ ) to 18, 25, and 9%, respectively. After 112 days, there was no difference ( $P > 0.05$ ) between NP and HP.

When evaluating the entire experimental period (from birth to slaughter), LP showed lower ( $P < 0.05$ ) ADFI (8%) and FCR (6%) than HP. Light BiW pigs need longer housing time (in the present study, 14 days more than NP and HP) and, consequently, higher cumulative feed intake (CFI, 7%) to achieve the same slaughter weight as NP and HP (Table 1).

Post-slaughter analyses were performed on pigs of same slaughter weight, which was reached at 188, 174, and 174 days for LP, NP, and HP, respectively (Table 2). Light BiW pigs presented lower ( $P < 0.05$ )

**Table 1** - *In vivo* analyses of performance of light (LP), normal (NP), and heavy (HP) birth weight pigs at 1, 28, 70, 112, and 174 days of age

Parameter	Birth weight category			SE	P-value
	LP	NP	HP		
Number of pigs	24	12	24	-	-
BW at birth (kg)	0.98c	1.42b	1.85a	0.012	0.000
28 days of age (weaning phase)					
BW (kg)	4.86c	6.40b	7.62a	0.163	0.000
ADG (kg)	0.20b	0.28a	0.32a	0.146	0.000
70 days of age (post-weaning phase)					
BW (kg)	24.95c	30.41b	33.33a	0.364	0.000
ADFI (kg)	0.66b	0.89a	0.90a	0.021	0.025
ADG (kg)	0.48b	0.57a	0.61a	0.016	0.000
FCR	1.37b	1.56a	1.48a	0.982	0.049
112 days of age (growing phase)					
BW (kg)	58.61b	69.41a	71.74a	0.697	0.000
ADFI (kg)	1.20b	1.90a	2.00a	0.053	0.000
ADG (kg)	0.81b	0.90a	0.91a	0.012	0.000
FCR	1.49b	2.11a	2.20a	0.059	0.000
174 days of age (finishing phase)					
BW (kg)	121.77b	137.09a	137.37a	1.390	0.000
ADFI (kg)	2.76a	2.96a	2.91a	0.272	0.002
ADG (kg)	1.08	1.10	1.06	0.017	0.557
FCR	2.56b	2.73ab	2.78a	0.301	0.000
At slaughter					
BW (kg)	139.68	137.07	137.42	0.220	0.0939
ADFI (kg)	2.26a	2.42ab	2.47b	0.220	0.000
ADG (kg)	0.97	1.02	1.00	0.070	0.999
FCR	2.33a	2.40ab	2.48b	0.200	0.050
CFI (kg)	277.1a	261.4b	258.1b	2.850	0.015
Age (days)	188	174	174	-	-

P - probability; SE - standard error; BW - body weight; ADFI - average daily feed intake; ADG - average daily gain; FCR - feed conversion ratio; CFI - cumulative feed intake.

a-c - Means followed by distinct letters within one row differ significantly by Tukey's test ( $P < 0.05$ ).

LEA (9.4%) and LMD (7.6%) values than NP and HP at the same slaughter weight. However, LP had higher ( $P<0.05$ ) BT (10.6%) and FA (11.8%) than HP.

Light BiW pigs had lower fat deposition, indicated by the lower ( $P<0.05$ ) M:F (21.6%) as compared with the other categories, producing lower ( $P<0.05$ ) CL, SF, and FI values than NP and HP. Light BiW pigs showed a lower total muscle fiber number ( $P<0.05$ ) than NP and HP. The centesimal analysis showed that the *longissimus thoracis* muscle in LP had higher ( $P<0.05$ ) gross energy levels (2.7%) and higher EE values (33.7%) than NP and HP (Table 3).

Light BiW pigs presented lower ( $P<0.05$ ) steak (7.0%) and belly + rib (8.6%) weights compared with NP and HP, which, in turn, did not differ among themselves (Table 4). The data for shoulder and shank weight did not show any significant difference between the BiW categories.

**Table 2 - Carcass characteristics and meat quality of light (LP), normal (NP), and heavy (HP) birth weight pigs at slaughter**

Parameter	Birth weight category			SE	P-value
	LP	NP	HP		
Carcass characteristics					
BW at slaughter (kg)	139.70	137.10	137.40	0.220	0.093
HCW (kg)	94.78	94.87	95.05	1.090	0.973
CCW (kg)	93.17	93.12	92.91	1.080	0.987
CY (%)	66.69	67.92	67.62	0.269	0.182
BT (mm)	21.74a	19.63ab	19.43b	2.980	0.019
FA (cm <sup>2</sup> )	25.57a	22.81ab	22.54b	2.850	0.001
LEA (cm <sup>2</sup> )	54.11b	61.62a	57.88a	0.837	0.004
LMD (mm)	67.83b	74.09a	72.84a	0.786	0.011
M:F	2.18b	2.84a	2.71a	0.085	0.004
YMCC (%)	57.13	57.59	57.43	0.529	0.937
Meat quality					
pH45	6.63	6.43	6.45	0.033	0.121
pH24	5.56	5.54	5.58	0.019	0.676
WHC (%)	15.77	14.07	14.01	0.888	0.607
CL (%)	19.87b	30.65a	29.57a	0.436	0.000
SF (N/kg)	8.06b	11.07a	11.81a	0.318	0.000
FI (N/mm)	2.85b	3.76a	3.62a	0.092	0.000

P - probability; SE - standard error; BW - body weight; HCW - hot carcass weight; CCW - cold carcass weight; CY - carcass yield; pH45 - pH in 45 min; pH24 - pH in 24 h; BT - backfat thickness at the 10th rib; FA - fat area; LEA - loin eye area; LMD - *longissimus thoracis* muscle depth; M:F - meat to fat ratio; YMCC - yield of meat in the cooled carcass; WHC - water holding capacity; CL - cooking loss; SF - shear force; FI - firmness.  
a-c - Means followed by distinct letters within one row differ significantly by Tukey's test ( $P<0.05$ ).

**Table 3 - Histological and centesimal analysis of the *longissimus thoracis* muscle of the left half carcass of light (LP), normal (NP), and heavy (HP) birth weight pigs at slaughter**

Parameter	Birth weight category			SE	P-value
	LP	NP	HP		
Histological analysis					
Fiber mean CSA ( $\mu\text{m}^2$ )	5.95	5.63	5.60	0.570	0.184
Total fiber number	603.70b	734.72a	735.50a	3.340	0.000
Centesimal analysis (Dry matter)					
Gross energy (kcal/kg)	5.36a	5.18b	5.26b	0.310	0.001
Crude protein (%)	76.54b	78.05ab	78.47a	0.240	0.001
Ether extract (%)	12.40a	8.83b	9.77b	0.330	0.000
Mineral matter (%)	4.11	4.23	4.33	0.100	0.585
Moisture (%)	94.81	94.50	93.55	0.290	0.134

P - probability; SE - standard error; CSA - cross-sectional area.  
a-c - Means followed by distinct letters within one row differ significantly by Tukey's test ( $P<0.05$ ).

**Table 4** - Weights of the primary cuts of the left half carcass of light (LP), normal (NP), and heavy (HP) birth weight pigs at slaughter

Parameter	Birth weight category			SE	P-value
	LP	NP	HP		
LCCW	46.67	46.3	46.17	0.527	0.904
	Yields of primary cuts				
Shoulder (kg)	10.11	9.86	9.90	0.72	0.565
Shank (kg)	16.62	16.18	16.08	1.82	0.065
Steak (kg)	9.71b	10.41a	10.37a	0.96	0.003
Belly + rib (kg)	20.65b	22.52a	22.34a	3.65	0.000

P - probability; SE - standard error; LCCW - left half cold carcass weight.

a-c - Means followed by distinct letters within one row differ significantly by Tukey's test ( $P < 0.05$ ). Using BW at slaughter as a covariate.

## 4. Discussion

The genetic selection of hyper-prolific sows caused an increase in the number of piglets born and greater variability in birth weight (Quiniou et al., 2002). Studies demonstrate BiW influences on growth performance, mortality, carcass characteristics, and meat quality of barrows and gilts pigs (Magnabosco et al., 2016; Huting et al., 2018; Lanferdini et al., 2018). Furthermore, with the increase of immunocastration as an alternative to surgical castration, there is little information on the effects of BiW in this category. Therefore, this study aimed to determine the effect of BiW on growth performance, carcass characteristics, and meat quality of immunocastrated male pigs.

Škorjanc et al. (2007) and Beaulieu et al. (2010) observed that LP presented lower BW throughout the suckling and nursery phases. Regardless of the nutrition and management practices adopted, LP remained light in the early stages of life. The lower postnatal development of LP can be attributed to their lower innate immune response and higher susceptibility to environmental challenges due to lower colostrum ingestion capacity, as well as the underdeveloped formation of muscle fibers in their prenatal life (Gondret et al., 2006; Rehfeldt and Kuhn, 2006; Huting et al., 2018).

Because the pigs in this study were genetically similar and experienced similar and favorable environmental conditions, differences in BW between the three categories were expected to remain the same throughout the experimental period (Alvarenga et al., 2013). Indeed, Rehfeldt et al. (2008) and Zhang et al. (2018) demonstrated that LP have lower growth rates during the postnatal period. However, the final BW of the HP category was not different than the NP, which may indicate that HP did not reach their maximum genetic potential. Studies have shown that heavier pigs at birth are heavier at slaughter (Rehfeldt et al., 2008).

The pigs used in this experiment had the same genetic background and, consequently, similar Gompertz equation parameters (A, B, C), so there was no reason to expect that the NP category would produce results similar to the HP category (Ceron et al., 2020). This aspect shows the need to develop nutritional and handling strategies to increase the performance of HP weight range, and not only to concentrate all the efforts of the farm to prioritize the survival and development of the LP. Actually, according to Gondret et al. (2006) and Rehfeldt and Kuhn (2006), LP need longer housing times, causing increase of CFI, production costs and can delay the slaughter age of the entire batch of animals. The influence of immunocastration on performance occurs after the application of the second dose of vaccine, and the largest effect is a drastic increase in feed intake (Batorek et al., 2012).

There are indications that BiW influences muscle fiber number and diameter (prenatal) and may compromise postnatal muscle development and interfere with carcass characteristics and meat quality (Alvarenga et al., 2013; Wang et al., 2017). Slaughter age can affect water loss through cooking, because younger animals have more moisture in their muscles, making their meat more susceptible to water loss at the time of cooking (Bonagurio et al., 2003). According to van Laack et al. (2001), body and intramuscular fat (IMF) decreases the values of shear force and firmness, which was evident in this

study, in which pigs with higher body fat presented lower water loss values. This may be associated with an increased growth rate in immunocastrated animals before slaughter, resulting in enhanced protein turnover *in vivo* and higher IMF content and lower SF (Batorek et al., 2012).

Another factor that interferes with water retention and correlates with meat quality is the number and size of muscle fibers (Rehfeldt and Kuhn, 2006). In this research, LP had the same sized fibers but fewer numbers, increasing water retention capacity for this weight range. The smaller number of muscle fibers presented by LP may be responsible for high FA and BT, and lower LEA, LMD, and M:F, at the time of slaughter (Rehfeldt and Kuhn, 2006; Alvarenga et al., 2013; Huting et al., 2018). Poore and Fowden (2004) in a study on LP and HP, found that the first had lower leptin content shortly after birth, but higher lipid deposition at 90 days. Another factor associated with greater fat deposition in LP may be associated with the level of circulating leptin. In immunocastrated animals, the circulating leptin levels at the second dose of vaccine are low, because they are leaner than castrated pigs (Batorek et al., 2012).

Centesimal analysis serves to assess nutritional characteristics of food. The higher EE value in the loins of the LP can be indicative of greater IMF because there is a high correlation between the loin fat content and IMF (Daszkiewicz et al., 2005). Higher IMF content is associated with greater succulence, palatability, and tenderness of meat (Watanabe et al., 2018). This may explain the SF and FI values in the LP, considering that lower SF and FI values are associated with better quality and greater consumer acceptance (Daszkiewicz et al., 2005).

Published data relating to BiW, with carcass characteristics and commercial cuts, are inconsistent, which can be attributed, in part, to differences in housing conditions, dietary strategies, sex category, and weight range of the various experimental designs (Pardo et al., 2013; Lanferdini et al., 2018). Some studies showed that HP have a higher weight of commercial cuts when compared with LP (Beaulieu et al., 2010; Alvarenga et al., 2013). This can be explained by the allometric growth of the cuts in relation to BW (Landgraf et al., 2006). However, when using slaughter weight as a covariate, it was found that BiW did not influence the weight of commercial cuts, except for the weights of the steak and belly + rib.

The lower weight of steak in LP is associated with lower LEA and the number of muscle fibers, which may be caused by nutritional restrictions in the prenatal period (Rehfeldt and Kuhn, 2006; Rehfeldt et al., 2008). Conversely, the lower belly and rib weight obtained for the LP is consistent with the results observed by Pardo et al. (2013), when compared with carcass kilograms.

In general, the results of the present study indicate that immunocastrated piglets with low BiW have low rates of postnatal growth, which negatively impacts carcass characteristics, although it does not greatly affect meat quality and weight of the commercial cuts of these animals. More studies are needed to confirm the influence of BiW on performance and carcass quality of immunocastrated pigs. Thus, the development of management strategies and feeding programs to maximize the deposition of lean meat and reduce the occurrence of piglets with low BiW are fundamental in the farming of immunocastrated pigs.

The objective of the present study was to evaluate the effect of BiW in immunocastrated pigs. The effect of weight and physiological age is evident in the outcomes. This fact causes some difficulties in interpretation, especially in the period after the second immunization. Therefore, we recognize this as one of the limitations of the present study and suggest that, in future studies, the inclusion of surgically castrated animals be considered.

## 5. Conclusions

Growth performance and carcass characteristics are influenced by the birth weight category. Light piglets require more time and feed intake to reach the same slaughter weight. Birth weight has positive effects on meat quality and weight of commercial cuts.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

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## Acknowledgments

The authors are grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for granting a doctoral scholarship to M.S. Ceron and for funding this project. We also thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting a master scholarship to M. Chimainski.

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